Attorney Docket No.: 6225.200-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Koch et al

Application No.: 10/016,858 Group Art Unit: 1617

Filed: December 14, 2001 Examiner: San Ming Hui

Confirmation No.: 7430

For: Hormone Composition

DECLARATION UNDER 37 C.F.R. 1.132 OF DR. LILA E. NACHTIGALL

I, Lila E. Nachtigall, declare as follows:

1. Since 1994, I have been a Professor in the Department of Obstetrics and Gynecology of New York University (NYU) School of Medicine and am also an Attending Physician at NYU Medical Center and Bellevue Hospital Center. I am also a Co-Director of Reproductive Endocrinology and Director of the bone Density Unit, both at NYU Medical Center. My major research interest has been in estrogen replacement in pre- and post-menopausal women, and I have written and lectured extensively on this topic, as reflected in my Curriculum Vitae (attached herewith as Exhibit A). I also have a substantial clinical practice in this therapeutic area.

2. I am not an inventor of the above-identified application. At the request of the assignee, I have studied the application and the Office Action of November 25, 2003. It is my understanding that the claims of this application, which are directed to the use of once-or-twice weekly administration of 10 ug estradiol via an intravaginal tablet to treat atrophic vaginitis, have been rejected as obvious over a series of references, i.e., Meignant (U.S. Patent 6,080,077), Mettler et al., *Maturitus* 14:23, 1991, and the Vagifem® monograph (describing the currently available 25 μg vaginal tablet). I further understand that the Examiner believes that Meignant teaches the local use of low dose estradiol, i.e., 2.5 or 5 μg, to treat atrophic vaginitis.

3. I disagree with the Examiner's conclusions regarding what practitioners in the field of hormone replacement therapy would have believed at the time the present patent application was filed. In my opinion, such practitioners, without access to the information disclosed in the Examples of the present application, could not have reasonably believed that that vaginal administration of a tablet containing 10 μg estradiol once or twice weekly would provide demonstrable clinical benefit for atrophic vaginitis. I base my opinion, at least in part, on the absence of clinical experience that could have supported a conclusion of efficacy of such a low dose of estradiol administered vaginally via a tablet.

4. The references cited by the Examiner, whether considered individually or taken together, would not have changed the view of a clinical practitioner discussed above. Specifically, the Meignant patent merely posits that low doses of estradiol can be administered using the particular soft-capsule dosage form described in the patent without resulting in significant systemic uptake; notably, however, there are no clinical data *at all* that relate to any effect on vaginal atrophy. For a clinician, this is a striking omission. The fact that the Meignant patent discloses that vaginal administration of the product does not result in increased plasma levels of estradiol does not speak to the issue of clinical benefit; conceivably, the lack of systemic uptake could also reflect an overall lack of *delivery* of estradiol to any tissues at all, much less the relevant tissues. (In other words, a demonstration of lack of serum uptake is not meaningful unless there is also an affirmative demonstration of a biological effect.) The so-called "clinical trials" described in Meignant could equally be taken as suggesting a *daily* dose of, e.g., 5 or 10 μg of estradiol; nothing in Meignant suggests using such low doses less frequently (such as, e.g., in a once- or twice-weekly dosing regimen).

5. Also relevant is the great deal of variation that exists among different dosage forms that can be used for intravaginal administration of drugs. It was well known, for example, that the use of estrogen creams results in much higher systemic uptake than does the use of tablets (such as, e.g., Vagifem®). In this context, Meignant does not relate to a tablet but to a soft capsule; accordingly, Meignant has only limited relevance for the use of tablets. It is also my understanding that soft capsules are inferior to tablets in that administration of soft capsules results in erratic release of their estradiol contents, in contrast with vaginal tablets which are know to release in a more constant manner.

6. Furthermore, our extensive experience with the current Vagifem® product (as reflected, e.g., in the Mettler et al. article and in the Vagifem® monograph) cannot be extrapolated to the use of a much lower dosage regimen as in the present invention. It was quite surprising that Vagifem® could provide clinical benefit; and -- in view of the absence of anything in the medical literature (or in anecdotal clinical experience, for that matter) that would have suggested that *further* lowering of the dosage by 40% or 80% would still provide enough estradiol locally to result in improvement of symptoms -- the invention described and claimed in the present application was even more surprising.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Lila E. Nachtigall, M.D. 2-23-04

Dr. Lila E. Nachtigall Date

CURRICULUM VITAE

Revised 11/06/03

NAME:	Lila E. Nachtigall, M.D.
HOME ADDRESS:	355 Riverside Drive, New York, New York 10025
OFFICE ADDRESS:	251 E. 33rd Street, New York, New York 10016
DATE OF BIRTH:	February 23, 1934
PLACE OF BIRTH:	New York City

EDUCATION:

1960	MD	New York Medical College, New York
1956	BS	Columbia University, New York
1955	BA	Brooklyn College, New York

POST-DOCTORAL TRAINING:

1964-1966	Obstetrics and Gynecology	Research Trainee, Bellevue Hospital Center New York University Medical Center, New York
1963-1964	Internal Medicine	Resident, Bellevue Hospital Center New York University Medical Center, New York
1963-1964	Radioisotopes, Medicine	Postgraduate, Columbia University College of Physicians and Surgeons, New York
1962-1963	Endocrinology	Fellowship, Bellevue Hospital Center New York University Medical Center, New York
1961-1962	Internal Medicine	Assistant Resident, Bellevue Hospital Center New York University Medical Center, New York
1960-1961	Medicine	Intern, Bellevue Hospital Center New York University Medical Center, New York

LICENSURE AND CERTIFICATION:

1965	American Board of Medical Specialists
1961	New York State, # 086367
1961	National Board of Medical Examiners, Diplomate

ACADEMIC APPOINTMENTS:

1994-Present	Professor	Obs/Gyn, Full-time New York University School of Medicine Department of Obstetrics and Gynecology New York, New York
1974-1994	Associate Professor	Obs/Gyn, Full-time New York University School of Medicine Department of Obstetrics and Gynecology New York, New York
1971-1974	Assistant Professor	Clinical Obs/Gyn, Part-time New York University School of Medicine Department of Obstetrics and Gynecology New York, New York
1968-1971	Assistant Clinical Prefessor	Part-time New York University School of Medicine Department of Obstetrics and Gynecology New York, New York
1966-1980	Clinical Instructor	Part-time New York University School of Medicine Department of Obstetrics and Gynecology New York, New York
1961-1964	Teaching Assistant	Part-time New York University School of Medicine Department of Medicine New York, New York

HOSPITAL APPOINTMENTS:

1999-Present	Attending	Bellevue Hospital Center Department of Obstetrics and Gyneclogy New York, New York
1975-1999	Assistant Attending	Bellevue Hospital Center Department of Obstetrics and Gynecology New York, New York

2000-Present	Attending	New York University Medical Center- Tisch Hospital Department of Obstetrics and Gynecology New York, New York
1974-2000	Assistant Attending	New York University Medical Center- Tisch Hospital Department of Obstetrics and Gynecology New York, New York
1999-Present		Co-Director Bone Density Unit New York University Medical Center- Tisch Hospital New York, New York
1973-1992	Director	Gynecologic Endocrinology Goldwater Memorial Hospital Department of Obstetrics and Gynecology Roosevelt Island, New York
1973-Present	Co-Director	Reproductive Endocrinology New York University Medical Center Tisch Hospital Department of Obstetrics and Gynecology New York, New York
1973-1998	Director	Gynecologic Endocrinology Outpatient Services Bellevue Hospital Center New York, New York
1966-1992	Consultant	Medical Endocrinology Goldwater Memorial Hospital Department of Obstetrics and Gynecology Roosevelt Island, New York
1965-1992	Assistant Attending	Goldwater Memorial Hospital Department of Obstetrics and Gynecology Roosevelt Island, New York
1964-1975	Assistant Visiting Attending	Bellevue Hospital Center Department of Obstetrics and Gynecology New York, New York

AWARDS AND HONORS:

2003	Award for Excellence, NYU Medical Center,Women's O.W.N.
2000	Achievement Award in Women's Health, ACOG
1999	International Society of Menopause, Award for Best Poster
1999	Distinguised Alumnus Award, Bellevue OB-GYN Society
1979	Founder's Award, Phi Sigma Sigma, Outstanding Alumnae
1960	Karl Harputer Award in Physical Medicine

MAJOR COMMITTEE ASSIGNMENTS:

New York University School of Medicine, New York City

1998-Present	Chair, Committe to Prevent Student Abuse
1998-Present	Member, Permenent Committee on Women's Issues
1994-Present	Member, Nominating Committee of Executive Board
1993-1998	Chair, The Joint Task Force on Women's Issues
1993-Present	Member, Clinical Education and Care Committee
1988-1998	Chair, Grievance Committee
1985-1993	Representative, Faculty Council
1984-1990	President, Parents Association
1978-Present	Member, Admissions Committee, NYU School of Medicine, New York City
1976-1998	Member, Quality Review Committee
1976-1991	Member, Utilization Review Committee

MEMBERSHIPS, OFFICES, AND COMMITTEE ASSIGNMENTS IN PROFESSIONAL SOCIETIES:

1995-Present	NYU School of Medicine GME Consotium-Primary Care Workgroup
1992-1999	The Bellevue Obstetrical and Gynecological Society, President
1991-Present	American Association of Clinical Endocrinologists
2000-2001	President-North American Menopause Society

1990-Present	The North American Menopause Society
1999-2000	President-Elect-The North American Menopause Society
1990-1992	The Bellevue Obstetrical and Gynecological Society, Vice President
1985-Present	New York Society of Reproductive Medicine
1985-Present	American Women's Medical Association
1985-1990	The Bellevue Obstetrical and Gynecological Society, Treasurer
1980-Present	American Society of Reproductive Medicine
1980-Present	New York Obstetrical Society
1970-Present	New York Medical Society
1968-Present	Endocrine Society

EDITORIAL BOARDS:

1995-Present	Editor, Menopause
1993-Present	Editor, Primary Care Update for Ob/Gyns
1997-Present	Editor-Menopause Management
1993-Present	Editor-in-Chief, International Perspectives on Menopause Management
1998-Present	Reviewer, Menopause Management
1990-Present	Reviewer, Endocrine Society Presentations
1989-Present	Reviewer, American Journal of Obstetrics and Gynecology
1989-Present	Reviewer, Journal of the American Medical Association
1986	Reviewer, The Physicians Video Guide
1985-Present	Reviewer, Medical Aspects of Human Sexuality
1975-1990	Advisor, PRN Radio—Editorial Board

MAJOR RESEARCH INTERESTS:

Estrogen replacement in pre- and post-menopausal women.

PRINCIPAL CLINICAL AND HOSPITAL SERVICE RESPONSIBILITIES:

1999-Present	Chair	Student Abuse Committee NYU School of Medicine New York, New York
1973-1992	Director	GYN-Endocrinology and Endocrinology Goldwater Memorial Hospital Roosevelt Island, New York
1976-1991	Member	Utilization Review Committee Bellevue Hospital Center New York, New York
1976-Present	Consultant	GYN-Endocrine Wards Residents and Fellows Bellevue Hospital Center New York, New York
1978-1999	Supervisor	GYN-Endocrine Clinic Residents and Fellows Bellevue Hospital Center New York, New York
1978-Present	Director	Reproductive Endocrine Conference(weekly) Third-year medical students, residents, and interested attendings. Bellevue Hospital Center New York, New York
1978-Present	Member	Admissions Committee New York University School of Medicine New York, New York
1980-Present	Consultant	GYN-Endocrine Residents Tisch Hospital of the New York University Medical Center New York, New York

1984-Present	Member	Quality Care Review Committee Department of Obstetrics and Gynecology Bellevue Hospital Center New York, New York
1985-1993	Member	Faculty Council New York University School of Medicine New York, New York
1988-1995	Co-Chairperson	Student Life Committee New York University Medical Center New York, New York
1991-Present	Member	Executive Committee of the Medical Board New York University Medical Center New York, New York
1991-Present	Chairperson	Women's Task Force New York University Medical Center New York, New York
1992-Present	Member	Student Abuse Committee New York University School of Medicine New York, New York

MAJOR ADMINISTRATIVE RESPONSIBILITIES:

1991-Present	Director	Women's Wellness Division Department of Obstetrics and Gynecology New York University Medical Center New York, New York
1985-1990	Co-Director	GYN-Endocrine Program New York University Medical Center New York, New York
1975-1999	Clinic Coordinator	GYN-Endocrine Clinic Bellevue Hospital Center New York, New York

TEACHING EXPERIENCE:

1971	Lecturer	*Symposium on Environment and Birth Defects* Commodore Hotel

New York, New York

1971 Lecturer — *Treatment of the Infertile Couple*
New York University Medical Center
Department of Urology
New York, New York

1971 Lecturer — *Complications Arising from Induction of Ovulation*
Booth Memorial Hospital
Flushing, New York

1972 Lecturer — *Induction of Ovulation*
American College of Obstetrics and Gynecology,
Nurses Association
New York, New York

1973 Lecturer — *"Genetic Aspects of Reproduction"* (Seminar)
Columbia College of Physicians and Surgeons
New York, New York

1973 Lecturer — *Placental Hormonology*
Lenox Hill Hospital
New York, New York

1974 Lecturer — *Endocrinology and Metabolic Diseases*
Joint Education Committee
Riverside, Dover's and St. Clare's Hospitals
New Jersey

1976 Lecturer — *Studies in Estrogen Use*
Panel on Estrogen in Menopause
New York Academy of Medicine
New York, New York

1976 Lecturer — *Estrogen Therapy in the Postmenopausal Era*
Advanced Seminar in Contraception Control and
Human Reproduction
New York University Medical Center
New York, New York

1977 Lecturer — *Recent Advances in Gynecologic Endocrinology*
Postgraduate course
University of Kentucky Medical Center
Kentucky

1978	Lecturer	*Menopause: Perspectives for Management in Bi-hormonal Cyclic Replacement Therapy— A Ten Year Prospective Study.* Eisenhower Medical Center Palm Desert, California
1978	Lecturer	*Menopause: Fact or Fiction?* Adult Education Program (Seminar) Bergen County Community College Paramus, New Jersey
1978	Lecturer	*Complications of Oral Contraceptives* Yale University School of Medicine New Haven, Connecticut
1978	Lecturer	*Nurse-Practitioner Conference: Family Planning* Gateway Hilton
1978	Lecturer	*Update on Contraception* University of Colorado Medical Center Boulder, Colorado
1979	Lecturer	*Course in Gynecologic Endocrinology to house staff* Columbia University College of Physicians and Surgeons, Harlem Division New York, New York
1979	Lecture	*Gynecologic Endocrinology*—Course for Section 13 New York State Medical Convention New York
1980	Lecturer	*Course in Osteoporosis and its Management* Oklahoma State Medical Association Oklahoma City, Oklahoma
1980	Conference Leader	*Estrogen Therapy* Bergen County Hospital Paramus, New Jersey
1980	Lecturer	*Anorexia Nervosa* Institute in Adolescent Medicine New York University Medical Center New York, New York

1980	Conference Member	*Prevention and Treatment of Osteoporosis* Emory University School of Medicine Lake Lanier, Georgia
1981	House Staff Conference	*Estrogens and Osteoporosis* Holy Name Hospital Teaneck, New Jersey
1981	Conference Leader	Visiting Professor University of Arizona School of Medicine Tucson, Arizona
1981	Lecturer	*Anorexia and Amenorrhea* Albert Einstein College Lecture series Norwalk Hospital Norwalk, Connecticut
1981	House Staff Conference	*Work-up and Treatment of Hirsutism in Women* New York Infirmary—Beekman Downtown Hospital New York, New York
1981	House Staff Conference	*Estrogens and Osteoporosis* Brookdale Medical Center Brooklyn, New York
1982	Lecturer	*Osteoporosis—Selected Updates in Obs/Gyn* New York Academy of Medicine New York, New York
1983	Course Instructor	*Reproductive Endocrinology Course* Metropolitan Hospital Center New York, New York
1984	Lecturer	*Polycystic Ovary Syndrome (general medical course),* Brookdale Medical Center Brooklyn, New York
1984	Grand Rounds	*Thyroid Disease in Pregnancy* Queens Hospital Jamaica, New York
1984	Lecturer	*Estrogen and Breast Cancer* National Institutes of Health—International Meeting Bethesda, Maryland

1985	Grand Rounds	*Pre-Menstrual Syndrome—An Update* Department of Obstetrics and Gynecology New York University Medical Center New York, New York
1985	Lecturer	*Transdermal Estrogen Substitution* XITH World Congress of Gynecology and Obstetrics International Symposium West Berlin, Germany
1986	Lecturer	*Another Morning on Menopause: Emotional Aspects of Menopause and New Hormone Treatment* The Mount Sinai Medical Center Department of Obstetrics and Gynecology New York, New York
1986	Lecturer	*Trans-cutaneous Estradiol (Estraderm) in the Management of the Climacteric Female* New York University Medical Center Department of Obstetrics and Gynecology New York, New York
1987	Lecturer	*New Advances in Estrogen Replacement Therapy (ERT)* Long Island City Hospital Long Island City, New York
1987	Lecturer	*Estraderm in the Current Management of ERT* New York University Medical Center— Grand Rounds Department of Obstetrics and Gynecology New York, New York
1987	Lecturer	*Hirsutism* NYU Medical Center—Endocrine Seminar Division of Endocrinology New York, New York
1987	Lecturer	*Estrogen Replacement* Women's Association for Research in Menopause First Annual Lecture Series Hunter College West New York, New York

1987	Lecturer	*The Menopause: Long-Term Experience with TTS Estradiol* CIBA Symposium Saint Thomas, Virgin Islands
1987	Lecturer	*Estrogen Replacement Therapy—An Update* Beth Israel Medical Center—Grand Rounds Department of Obstetrics and Gynecology New York, New York
1987	Lecturer	*International Symposium on Transdermal Estradiol Substitution* 5th International Congress on the Menopause Sorrento, Italy
1987	Lecturer	*Management of the Climacteric in 1987* American College of Obstetricians and Gynecologists 35th Annual Meeting Las Vegas, Nevada
1987	Lecturer	*A Review of Estrogen Replacement Therapy (ERT)* North Shore Medical Center—Grand Rounds Department of Obstetrics and Gynecology Manhasset, New York
1987	Guest Speaker	*Estrogen Replacement Therapy in the 80s* The 32nd Annual Raymond M. Kay, M.D. International Medicine Symposium Kaiser Permanente—Southern California Permanente Medical Group Los Angeles, California
1989	Lecturer	*Multidisciplinary Perspectives on Menopause* The New York Academy of Sciences The North American Menopause Society The Sheraton Centre Hotel New York, New York
1990	Visiting Professor	*Update on Estrogen Therapy* University of Florida Department of Obstetrics and Gynecology Gainsville, Florida

1993 Lecturer — *Hormone Replacement Therapy and Breast Cancer*
The Society of Alumni of Bellevue Hospital
The Bellevue Hospital
Department of Obstetrics and Gynecology
New York, New York

1993 Lecturer — *Hormone Replacement Therapy.*
The Blue Ribbon Women's Health Seminar, The Music Center of Los Angeles County. Los Angeles, CA

1993 Lecturer — *Clinical Aspects of Dysfunction of the Female Reproductive System,* Department of Cell Biology at New York University Medical Center, NY.

1993 Lecturer — *Compliance with Hormone Replacement Therapy: Where We Stand Today.* 7th International Congress on the Menopause. Stockholm, Sweden.

1993 Lecturer — *Evaluation of a bioadhesive vaginal moisturizing gel and Premarin cream in the treatment of vaginal dryness postmenopausal women.* Abstract 403 for poster session in 7th International Congress on the Menopause, Stockholm, Sweden.

1993 Lecturer — *Breast Cancer Issues*, North American Menopause Society meeting, San Diego, CA.

1993 Lecturer — *Realities of Mid-life in Women: Gynecological Considerations*, Novo-Nordisk Pharmaceutical Symposium. Copenhagen, Denmark.

1993 Lecturer — *Prevention of Ovarian Dysfunction,* The American Fertility Society, 49th Annual Meeting, Montreal, Quebec, Canada.

1993 Lecturer — *ERT in Postmenopausal Women*, Grand Rounds at New York Hospital Medical Center, Flushing, NY.

1993 Lecturer — *Estrogen and Breast Cancer and other Concerns*, Wyeth Pharmaceutical guest speaker at United Hospital Medical Center, Portchester, NY.

1993 Lecturer — *Osteoporosis: Basic Science and Clinical Care*, American Women's Medical Association, Marriot Marquis Hotel, NY.

1993	Lecturer	Osteoporosis Master Faculty Update, Annual Meeting of the American Women's Medical Association, Marriot Marquis Hotel, NY.
1993	Lecturer	Upjohn Visiting Professor, Australia and New Zealand.
1993	Lecturer	*Estrogen and Breast Cancer*. Lecture for the Postgraduate Medical Committee in the University of Auckland, sponsored by Upjohn. Auckland, New Zealand.
1993	Lecturer	*HRT and the Cardiovascular System*, Consensus Conference for the American Fertility Society's Workshop on "Progestins and Androgens: Compliance Issues", Bethesda, MD.
1993	Lecturer	*Menopause,* lecture to third-year students at New York University School of Medicine, NY.
1993	Lecturer	*Amenorrhea*, lecture for Resident Didactic Series at the Bellevue Hospital, NY.
1993	Lecturer	*ERT and Osteoporosis,* lecture to Medical House Staff at the Bellevue Hospital, NY.
1993	Lecturer	*ERT and Osteoporosis,* lecture to Medical House Staff at the New York University School of Medicine, NY.
1994	Lecturer	*Endocrine Disorders*, lecture to third-year students at the Bellevue Hospital NYC.
1994	Lecturer	*The Use of Estrogen Replacement in the Compromised Patient*, Danbury Hospital, Danbury, CT.
1994	Lecturer	*The Peri-menopause*, lecture to physicians at the Ramada Hotel, NYC.
1994	Lecturer	*Menopause*, lecture to third-year students at the Bellevue Hospital, NYC.
1994	Lecturer	*Menopausal Syndrome/Estrogen /Androgen Therapy,* Grand Rounds, Maimonides Hospital, Brooklyn, NY.

1994	Lecturer	*ERT with Emphasis on Compliance and Breast Cancer,* Grand Rounds, North Shore Hospital, NY.
1994	Lecturer	*Amenorrhea,* lecture to third-year students at the Bellevue Hospital, NYC.
1994	Lecturer	*Horrmone Replacement Therapy,*Grand Rounds at Bon Secours Hospital, Detroit, MI.
1994	Lecturer	*Dysfunctional Uterine Bleeding—The Perimenopausal Patient*, OB/GYN Grand Rounds at St. Vincent's Hospital, NYC.
1996	Lecturer	*Should Every Woman Be On Hormone Replacement Therapy,* Winter Scientific Seminar, Kansas City, MO
1996	Lecturer	*The Role of Hormone Replacement Therapy In: Breast Cancer,* Women's Health Forum, North Shore University Hospital, Manhasset, N.Y.
1996	Lecturer	*Towards Better Recognition of Urogenital Ageing*, 8th Internaltional Congress on the Menopause. Sydney, Australia.
1997	Guest Speaker	*Hormone Replacement and Osteoporosis*, The Sixth Women's Health Update: Emotional & Physical Health. Ledyard, Connecticut.
2001	Lecturer	*Menopause & HRT*, lecture to third-year students at the Bellevue Hospital, NYC.
2002	Guest Speaker	*Hormone Replacement Therapy and Cardiovascular Disease*, Baptist College of Health Sciences, Memphis, TN.
2002	Lecturer	*Menopause & HRT*, lecture to third and fourth-year students at the Bellevue Hospital, NYC.
2002	Guest Speaker	*Lipids Management and Treatment in Women*, Baptist College of Health Sciences, Memphis, TN.
2002	Lecturer	*Boning Up On Osteoporosis*, New York University School of Medicine, NYC.
2003	Course Director	*Council on Hormone Education*, Scottsdale, AZ.

2003 Lecturer	*Menopause & HRT*, lecture to third and fouth-year students at the Bellevue Hospital, NYC.
2003 Board Member	*Advisory Board on SERMS*, Beaver Creek, CO.

BIBLIOGRAPHY:

Original Reports:

1. Nachtigall LE, Basset M, Hogsander U and Slagle S. A rapid method for the assay of plasma estriol in pregnancy. J Clin Endocr Metabolism 1966; **26**(9):491.

2. Nachtigall LE, Bassett M and Levitz M. Plasma estriol levels in normal and abnormal pregnancies. An Index of Fetal Welfare 1968; **101**:683.

3. Jewelewicz R and Nachtigall LE. Pseudo-pseudohypoparathyroidism and pregnancy. Obstetrics and Gynecology 1971; **37**:396.

4. Rifkin I, Nachtigall LE and Beckman EM. Amenorrhea following oral contraceptives. Am J Obs Gyn 1972; **113** (3):420.

5. Beller FK, Nachtigall LE and Rosenberg M. Coagulation studies of menopausal women taking estrogen replacement. Obstetrics and Gynecology 1972; **35**(5)

6. Weiss G, Nachtigall LE and Ganguly M. Induction of an LH surge with estradiol benzoate. A clinical test of pituitary-hypothalmic axis competence. Obstetrics and Gynecology 1976(Apr);**47**(4):415.

7. Nachtigall LE. Estrogen: Friend or Foe? Health Digest, 1976 (May).

8. Nachtigall LE, Nachtigall RH, Nachtigall RD, et al. Estrogen therapy and endometrial carcinoma: Correspondence. New England Journal of Medicine 1976; **294**:848.

9. Nachtigall LE. Hormones of the menstrual cycle. In: A Blaustein (ed.). Pathology of the Female Genital Tract. New York: Springer-Verlag, 1977.

10. Nachtigall LE, Nachtigall RH, Nachtigall H and Beckman EM. Estrogen replacement therapy I: A ten-year prospective study in the relationship to osteoporosis. Obstetrics and Gynecology 1979; **53**:277.

11. Nachtigall LE, Nachtigall RH, Nachtigall RD and Beckman EM. Estrogen replacement therapy II: A ten-year prospective study in the relationship to carcinoma and cardiovascular and metabolic problems. Obstetrics and Gynecology 1979; **54**:74.

12. Szlachter B, Nachtigall LE, et al. Premature menopause: A reversible entity? Obstetrics and Gynecology 1979; **54**:396.

13. Nachtigall LE. A review of Sheehan's syndrome. Hospital Syndrome 1980 (Sept).

14. Nachtigall LE. Menopause and hysterectomy. Medical Aspects of Human Sexuality 1980; **14**:94.

15. Nachtigall LE. Answers to questions. Medical Aspects of Human Sexuality 1981; **15**:19.

16. Nachtigall LE. Evaluation of the hirsute female. Hospital Medicine 1982 (Feb).

17. Nachtigall LE. The biochemistry of adrenal virilization. Hospital Medicine 1984 (Sept):143.

18. Nachtigall LE and Nachtigall RD. Evaluating the newly menopausal woman. Contemporary OB/GYN 1985 (May):68.

19. Hammond CD, Nachtigall LE. Is estrogen replacement therapy necessary? Journal of Reproductive Medicine 1985(Oct); **30**(10 Suppl):797.

20. Kaplan NM, Mishell DR Jr, Hammond CB, Henderson BE, LaRosa JC, Lobo RA Mashchak CA, Nachtigall LE, Perry HM Jr, and Ross R. Management of the postmenopausal woman with hypertension. Case presentation and panel discussion. Journal of Reproductive Medicine 1985 (Oct); **30**:821.

21. Nachtigall LE. Thromboembolic risk with oral contraceptives. Consultant 1985 (Dec) **25**(18):19.

22. Nachtigall LE. Prevention and treatment of osteoporosis. Management of the Post-menopausal Patient Syllabus. American College of Obstetricians and Gynecologists 1986:103.

23. Nachtigall LE. Sexual activity in older women. Management of the Post-menopausal Patient Syllabus. American College of Obstetricians and Gynecologists 1986:135.

24. Nachtigall LE and Utian W. Comparative efficacy and tolerability of transdermal estradiol and conjugated estrogens. In: C Lauritzen (ed.). Transdermal Estrogen Substitution. Bern: Honsttuber, 1987.

25. Nachtigall LE. Cardiovascular disease and hypertension in older women. OB/GYN Clinics of North America 1987(Mar); **14**(1):89.

26. Nachtigall LE. Estrogen replacement: Which post menopausal women will benefit? The Female Patient 1987(Aug); **12**(8):72.

27. Arny M, Nachtigall LE, and Quagliarello JR. The effect of preimplantation culture conditions on murine embryo implantation and fetal development. Fertility and Sterility 1987 (Nov);**48**:5.

28. Nachtigall LE. Post menopausal related health problems. Family Medicine Update 1988(Mar); New York: State University of New York at Stony Brook.

29. Nachtigall LE, Gambrell RD Jr, Notelovitz M. Transdermal estrogen pros and cons. ACOG Update 1989 (May); **14**:11.

30. Nachtigall LE. Severe polyglandular disease: Case challenge. Menopause Management 1989; **II**:1.

31. Nachtigall LE. Enhancing patient compliance with hormone replacement therapy at menopause. Obstetrics and Gynecology 1990; **75**:77S(Suppl).

32. Nachtigall LE and Nachtigall LB. Protecting older women from their growing risk of cardiac disease. Geriatrics 1990(May); **45**(5):24.

33. Goldstein SR, Nachtigall MJ, Snyder JR and Nachtigall LE. Endometrial assessment by vaginal ultrasonography before endometrial sampling in patients with post menopausal bleeding. American Journal of Obstetrics and Gynecology 1990;**163**:116.

34. Kable WT, Gallagher JC, Nachtigall LE and Goldgar D. Lipid changes after hormone replacement therapy for menopause. Journal of Reproductive Medicine 1990(May); **35**:5.

35. Nachtigall LE, Nachtigall MJ. The perimenopause and vasomotor symptoms. In Postgraduate Medicine—A Special Report, August 22, 1990; McGraw-Hill Healthcare Publications, Minneapolis.

36. Nachtigall LE. The menopause. Conn's Current Therapy. New York: Saunders,1991.

37. Nachtigall MJ, Smilen SW, Nachtigall RD, Nachtigall RH, and Nachtigall LE. Incidence of breast cancer in a 22-year study of women receiving estrogen-progestin replacement therapy. Obstetrics and Gynecology 1992 (Nov); **80**:827.

38. Nachtigall LE and Nachtigall MJ. Hormone replacement therapy. Current Opinion in Obstetrics and Gynecology 1992 (Dec); **4**: 907.

39. Nachtigall LE. Compliance with hormone replacement therapy: Where we stand today, Chapt. 57. 7th International Congress on the Menopause in Stockholm, Sweden. UK:Plenum, 1993.

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Treatment of urogenital atrophy with low-dose estradiol: preliminary results

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ABSTRACT

Objective: To determine the lowest dosage of vaginally administered estradiol (E_2) that reverses signs and symptoms of urogenital atrophy but does not substantially increase plasma E_2 levels.

Design: Single-blind, single-arm study to determine the effects of de-escalating doses of vaginal estrogen on symptoms of urogenital atrophy, vaginal pH, and vaginal and urethral cytology. A questionnaire was used to assess subjective vaginal and urethral symptoms. Objective measurements included vaginal and urethral cytology, pH, endometrial biopsy, and 24-h circulating plasma luteinizing hormone, follicle-stimulating hormone (FSH), E_2, and estrone levels obtained in a Clinical Research Unit. Circulating E_2 levels were assayed with an ultrasensitive yeast bioassay with a detection limit of 0.02 pg/mL. Measurements were obtained over a 24-h period after administration of vehicle alone, on day 1 after the initial vaginal E_2 dosage, after 3 weeks of daily E_2 administration, and after an additional 9 weeks of twice weekly administration.

Results: From the first seven subjects studied at a 10-μg dose of E_2, 100% responded according to predefined criteria. Vaginal cytology showed statistical improvement at 3 and 12 weeks. Urethral cytology was statistically improved after 12 weeks. Vaginal pH decreased from postmenopausal to premenopausal levels at both 3 and 12 weeks. Eighty-two percent of symptoms were cured or improved. Endometrium remained atrophic. Circulating E_2 levels remained within the postmenopausal range of 3–10 pg/mL.

Conclusion: A 10-μg dose of vaginal E_2 effectively treated urogenital atrophy in seven women and did not cause endometrial hyperplasia or increase E_2 levels.

Key Words: Vaginal estradiol – Urogenital atrophy – Urogenital disease – Low-dose estrogen – Ultrasensitive estradiol bioassay.

Screening mammography allows earlier detection of breast cancer and initiation of treatment before development of regional or distant metastases. For this reason, a greater number of women are cured of this disease and become long-term survivors after initial diagnosis. At the time of breast cancer diagnosis, the majority of women are menopausal. In those who are premenopausal, adjuvant chemotherapy frequently induces permanent ovarian failure. Accordingly, a large and increasing number of breast cancer survivors are chronically exposed to low levels of estrogen and experience symptoms caused by lack of this hormone.

At the present time, many consider the use of estrogen replacement therapy (ERT) to be relatively contraindicated in survivors of breast cancer.[1] Although not substantiated in clinical trials, ERT could theoretically increase the risk of a second primary or stimulate the growth of occult metastases from the original tumor.[2] Others consider that ERT may be used in such patients,

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based on data obtained in observational studies.[2] Although the risk of ERT in these patients has not been established, the majority of survivors of breast cancer are not willing to take ERT.[3] Consequently, alternatives to ERT are needed for relief of menopausal symptoms and prevention of osteoporosis and heart disease.[4]

A major problem related to menopause is the development of urogenital atrophy.[5–7] The vagina, vulva, urethra, and trigone of the bladder all contain estrogen receptors and undergo atrophy when estrogen levels decrease. The vulva and the vaginal walls also become pale and thin and lose their elasticity. This results in decreased vaginal secretion and susceptibility to trauma and pain. In addition, the estrogen-deficient vagina develops an alkaline pH ranging from 5.5 to 6.8,[5,6] which increases the likelihood of urinary tract infections.[7] From 50% to 75% of breast cancer survivors indicate on questionnaires that they experience one or more symptoms of urogenital atrophy.[8] Symptoms include vaginal dryness, dyspareunia, urinary frequency, repetitive urinary tract infections, or urinary incontinence. Dyspareunia leads to decreased interest in coitus. As frequency of coitus diminishes, vaginal lubrication declines further.[9,10] These symptoms are not adequately resolved by using vaginal moisturizers or water-soluble lubricants[11–13] but are relieved by local estrogen application into the vagina.

Most recommended regimens of vaginal estradiol (E_2) markedly increase plasma estrogen levels.[14] We postulated that it might be possible to lower the vaginal E_2 dose to one which does not increase plasma E_2 levels but still effectively matures vaginal and urethral mucosa. We designed this study to develop a method to relieve symptoms of urogenital atrophy in breast cancer survivors without increasing systemic levels of estrogen. Our strategy was to determine the minimal effective dose of vaginal E_2 that would reverse urogenital atrophy.

Doses of vaginal E_2 cream currently recommended by the manufacturer range from 100 to 500 μg/day,[15] 10-fold higher than may be necessary based on the literature.[16,17] The lowest dose of vaginal E_2 reported to be effective for treating vaginal atrophy is 5 to 10 μg/day, delivered via a silastic ring.[18–22] The next lowest effective dose is a daily 10-μg vaginal tablet.[23,24] Accordingly, for this study, we chose a starting dose of 10 μg daily, to be reduced to a frequency of twice weekly after 3 weeks of administration. In the later phases of the trial, doses of 5, 2.5, and 1.25 μg are planned to be given on the same schedule.

A major limitation of examining systemic absorption from low-dose vaginal E_2 is the sensitivity of currently available E_2 radioimmunoassays (RIAs). The detection limit for most RIAs is 10 to 20 pg/mL.[25–28] Basal levels of E_2 when measured by the most sensitive RIAs range from 3 to 10 pg/mL. Consequently, assays of even higher sensitivity are required to detect small increments in plasma E_2 expected after low-dose estrogen administration. For these studies, we employed an ultrasensitive bioassay for E_2, which had been validated for measurement of E_2 in prepubertal girls and boys. This bioassay has a level of sensitivity 50- to100-fold greater than that of current RIAs and can detect levels in plasma as low as 0.02 pg/mL.[29–31]

In this article, we report preliminary findings from a study designed to identify the minimal effective dose of vaginal E_2 in postmenopausal women. As primary endpoints, we examined patient self-report (urogenital symptom index) as well as clinician-observed and laboratory parameters of urogenital atrophy. We chose to include only postmenopausal women without a history of breast cancer to prove safety and efficacy before use in survivors of breast cancer. Our preliminary results describe responses in the first group of seven patients treated for 3 months. We demonstrate that 10 μg of vaginal E_2, when given twice weekly, produce objective normalization of vaginal atrophy and relief of symptoms with only minimal increments in plasma E_2.

METHODS

Subjects

Symptomatic postmenopausal women without a history of breast cancer were selected for study according to the following criteria: (1) regular menses had ceased at least 2 years before beginning of study, or bilateral oophorectomy was performed at least 2 years before beginning of study; (2) one or more symptoms of estrogen deficiency; (3) laboratory signs of urogenital atrophy to include either <10% superficial cells on vaginal or urethral smear or vaginal pH > 5.5; and (4) luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in the postmenopausal range. Exclusion criteria included the following: (1) history of uterine cancer; (2) vaginal bleeding of unknown origin; (3) acute or chronic liver disease; (4) history of deep venous thromboembolic disease or pulmonary embolism; (5) use of sex hormone medications within past 3 months; (6) grade II–III uterovaginal prolapse; (7) chronic use of steroids, phenytoin, or p450 metabolized medications; (8) gallstones; (9) endometrial proliferation by biopsy; or (10) hysterectomy.

Evaluation of symptoms

A urogenital symptom questionnaire was developed to evaluate the occurrence and impact of symptoms of hypoestrogenic urogenital atrophy and response to therapy. This instrument presented questions regarding the severity and frequency of eight symptoms during the 7 days preceding the visit. Severity was graded as mild, moderate, severe, or very severe. Frequency of occurrence was recorded as not at all, a little bit, somewhat, quite a bit, or very much. The eight questions asked were the following: (1) Do you urinate too frequently during the day? (2) Do you have a feeling of urgency (need to empty your bladder)? (3) Do you have urine leakage related to urgency (immediate need to empty your bladder)? (4) Do you have pain or burning with intercourse? (5) Do you have feelings of vaginal dryness? (6) Do you have itching or burning with urination? (7) Do you have itching or burning outside the vagina? (8) Do you have pain with intercourse?

Definition of responses

Classification of a patient as a responder required both patient self-report and clinician-observed improvement. Symptomatic improvement required a change in the severity of at least one symptom by at least one grade. Clinician-observed improvement required a fall in the pH by at least 0.5 units. In addition, one step of improvement of at least one grade using the previously published Vaginal Health Index (VHI) needed to be documented.[13,32] The VHI involved use of vaginal and urethral cytology and calculation of the vaginal maturation index. An observer blinded to the study design evaluated vaginal/urethral cytology to determine the percentage of superficial, parabasal, and basal cells present. Improvement in the vaginal or urethral mucosa required a change in the maturation value of at least 10 points compared with baseline. Normal ranges published by Mandel et al.[33] were used to determine whether patients had achieved normal levels of vaginal superficial cells by the third month of treatment.

Vaginal E_2 doses

The study protocol is designed to enter groups of seven women each at one of several doses of vaginal E_2 (i.e., 10, 5, 2.5, 1.25 μg). If 80% of women respond to the higher dose, the next group is to be treated with the next lower E_2 dosage. If the group receiving 1.25 μg of E_2 responds, the final group is to be treated with vehicle as a placebo in an identical fashion to those receiving medication. If <80% of women respond to a given dose, the study will be terminated. Lack of response to a given dose will obviate the need for a placebo group. The current report includes only patients treated with the 10-μg dose. Informed consent from the patients did not indicate when in the E_2 dosage order a placebo would be used; therefore, women were blinded to the therapy received (single-blinding).

Treatment components

The study is divided into three components: a priming component (daily administration of vaginal E_2 for 3 weeks), an acute maintenance component (twice weekly administration for 9 weeks), and a chronic maintenance component (continuation of the acute maintenance dose for an additional 40 weeks or total of 1 year).

Vaginal E_2 formulation

The emollient base used by the manufacturer to prepare Estrace vaginal cream was used to dilute Estrace from a concentration of 100 μg/g to 10 μg/g. This preparation was prepared by our research pharmacist and inserted into a tube with a graded applicator identical to that used for Estrace.

Baseline biochemical studies

A complete blood count, multiphasic screen, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were obtained at baseline to ensure that inclusion criteria were met.

Baseline clinical studies

A pelvic examination was performed with determination of vaginal pH and maturation index and observation for evidence of estrogen deficiency, including pallor, friability, petechiae, and dryness. These parameters were each graded from 1 (poorest) to 5 (best) using the VHI. Endometrial biopsy was obtained with a tubular plastic device (Pipelle sampling device). Endometrial thickness was evaluated using a GE 3200 ultrasound equipped with a 7.0 MHz transvaginal microconvex probe. Urethral smears were obtained with a nylon brush (Cytobrush).[34] A score was assigned to pH values as shown in Table 1.

Study procedures

After two preliminary outpatient visits for physical examination, endometrial biopsy, and blood drawing, patients were admitted to the General Clinical Research Center (GCRC) to assess the absorption of vaginal E_2.

TABLE 1. *Vaginal pH scores*

Score	pH
5	≥6.1
4	5.5–6.0
3	5.0–5.4
2	4.5–4.9
1	≤4.4

At 6:00 AM, a venous cannula was placed in a forearm vein, and blood samples for basal and serial E_2, estrone, LH, and FSH were drawn. Vaginal vehicle for the E_2 was administered at 7:00 AM (placebo). Blood samples were obtained every 4 h for a 24-h period. During the next study day, the procedure was identical except that the patient received 10 μg of vaginal E_2 or placebo at 7:00 AM. Each patient then received a tube of vaginal E_2 or placebo that equaled the initial dose that was to be taken daily for 3 weeks (priming dose).

After 3 weeks, each woman was readmitted for 1 day to evaluate subjective and clinical improvement as well as signs of systemic effects, and the same protocol for frequent blood sampling was followed. The patient then decreased the frequency of her dose to the acute maintenance dose of 10 μg twice a week for 9 weeks. Compliance with taking medication was assessed by weighing the tube containing E_2 at each visit and comparing this weight with the starting weight of the filled tube minus the original empty weight of the tube. With this method, compliance was excellent. The average weight of the medication used was 43.1 g with an absolute range of 36.7 to 48.2 g, representing a variance of ±15%.

Patients were evaluated again in the GCRC after 9 weeks with a protocol identical to that at 3 weeks. In addition, an endometrial biopsy was obtained to assess for stimulation of endometrial proliferation. If a response were maintained and there were no adverse effect on the endometrium, the chronic maintenance phase began. The study plan has patients continuing the same dose twice a week for an additional 40 weeks. This portion of the study has not yet been completed.

Biostatistical analysis

Comparison of 3-week and 12-week assessments to baseline symptom scores, vaginal cytology, and urethral cytology were based on the Friedman's rank test, using the multiple comparisons procedure in Hollander et al.[35] Repeated measures models were used to compare E_2, estrone, and FSH and LH levels over time and across visits.

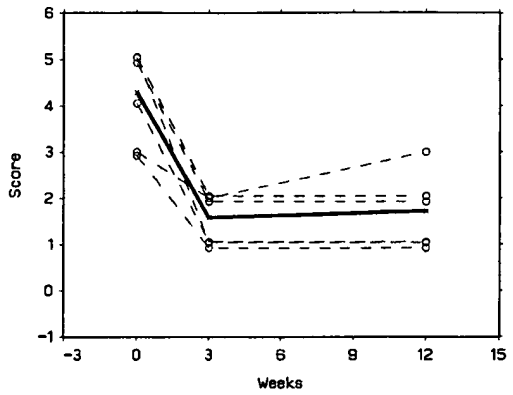

FIG. 1. Vaginal pH scores by patient and week. The dark solid line is the average pH at each follow-up time. The lighter lines are individual patient profiles. The 3- and 12-week scores are significantly different from the baseline scores ($p < 0.01$).

Estrogen assays

Serum E_2 was measured with an ultrasensitive yeast bioassay with a sensitivity of 0.02 pg/mL. This assay uses a strain of *Saccharomyces cerevisiae* genetically engineered for extreme sensitivity to estrogen by transformation with plasmids encoding the human estrogen receptor and two estrogen response elements linked to the β-galactosidase reporter. The sensitivity, precision, cross-reactivity with other steroids, correlation with RIA, and validation in menopausal patients have been previously published.[29–31] At a plasma concentration of 2 pg/mL, the coefficient of variation is 15%. Plasma estrone measurements involved a previously published RIA.[36] This assay is maximized for sensitivity based on choice of antibody and use of 4 mL of serum for extraction and specificity based on use of celite column and antibody. This assay can distinguish 5 pg/mL of estrone from blank with 95% confidence limits. The within assay coefficient of variation is 4.3%.

RESULTS

Clinical responses

All seven patients were classified as responders based on the objective and subjective criteria defined in the methods section. For all patients, vaginal pH scores were, on average, 2.7 categories better after 3 weeks of treatment than at baseline ($p < 0.01$). These improvements were maintained through the 12-week assessment period (Fig. 1). The number of superficial cells on vaginal smear (Fig. 2A) increased in all patients. Mean superficial cell percentage increased at 3 weeks ($p < 0.05$) and, by 12 weeks, reached levels midway between early (5%) and late (45%) follicular levels in nor-

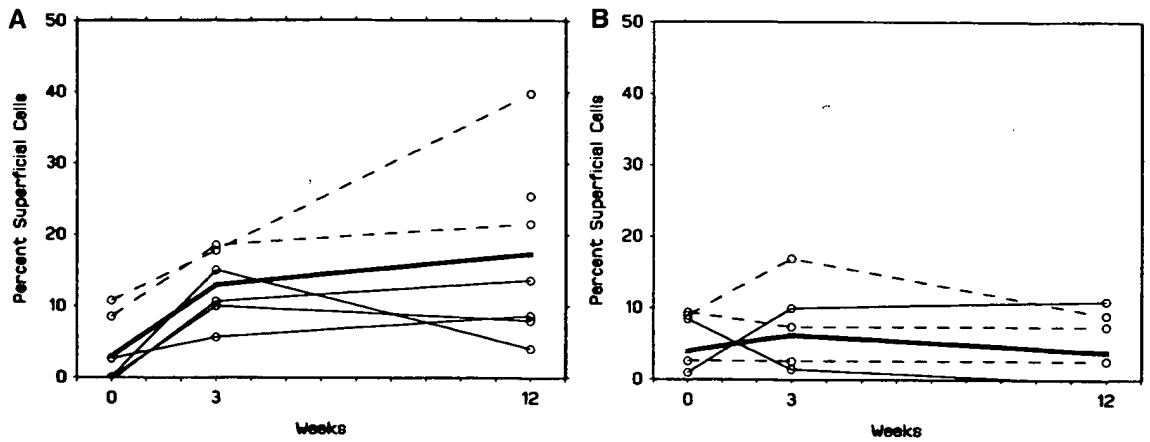

FIG. 2. Vaginal and urethral cytology, percent of superficial cells. The dark solid line is the average at each follow-up time. The lighter lines are individual patient profiles. **A:** % vaginal superficial cells by patient and week. The % superficial cells at weeks 3 ($p = 0.03$) and 12 ($p = 0.01$) are significantly different from baseline. **B:** % urethral superficial cells by patient and week. The % urethral superficial cells at baseline, 3, and 12 weeks are not significantly different ($p = 0.84$).

mal women ($p < 0.05$).[33] The two subjects with parabasal cells at baseline had no detectable parabasal cells at the end of 12 weeks (data not shown). Urethral superficial cells showed slight increases (p = ns) over time (Fig. 2B), whereas urethral parabasal cells showed marked individual variability after initial decreases from 60% to 20% at 3 weeks.

Symptomatic improvement

Of a total of 22 symptoms noted at baseline in the seven patients, 18 (82%) improved by at least one grade at the end of 3 months of treatment. (Fig. 3A). The total symptom score improved from 5.1 ± 1.4 to 1.4 ± 0.9 (Fig. 3). Symptoms of urethral atrophy improved in 100% of women receiving the 10-μg doses of E_2 (Fig. 3). Symptoms of vaginal atrophy improved in three of seven (42%) subjects receiving this dosage (Fig. 3). When patients were switched from the priming dose of 10 μg daily to 10 μg twice weekly (after week 3), symptoms did not worsen but continued to improve (Fig. 3). When responses were scored as to the bothersome nature of symptoms (Fig. 4), baseline total scores were 5.1 ± 0.7 and, after 3 months of treatment, were 0.9 ± 0.6.

Endometrial stimulation

Vaginal E_2 could potentially stimulate the endometrium and induce endometrial carcinoma. For this reason, endometrial biopsies were performed. No patient exhibited endometrial stimulation on repeat endometrial biopsy after 3 months of low-dose vaginal E_2, and no subject had a thickening of the endometrial stripe on ultrasound beyond 5 mm.

Plasma estrogen levels

Estradiol

Measurement of E_2 levels after administration of placebo cream revealed levels averaging 1 to 3 pg/mL (Fig. 5A). These concentrations are substantially below those usually detected by RIA (i.e., 5 to 20 pg/mL) but are consistent with previous measurements in postmenopausal women and early pubertal girls.[30,31] Levels were relatively constant during the 24-h period. If a diurnal pattern was present, the increase did not exceed 0.5 pg/mL. After the initial insertion of vaginal cream, plasma E_2 levels began to increase at 1 h and peaked at 4 h before returning to baseline at 8 h. Peak levels reached 3.8, an increment of 1.8 pg/mL over basal levels of 2 pg/mL. After insertion of E_2 cream at 3 weeks and again at 3 months, the patterns of increase were similar. Although absorption seemed to be greater at 3 months than basally, this change was not statistically significant. At each study period with patients receiving E_2, basal (time 0) levels of E_2 were never higher than levels before administration of E_2 during the first GCRC visit. During chronic administration of low-dose vaginal E_2, levels of this sex steroid were elevated by an average of only 2 pg/mL and only during the first 4 h after administration of E_2. Consequently, during the 72 to 96 h between applications (i.e., twice weekly), minor elevations of E_2 were present only for 4 h.

Previously published studies report an average metabolic clearance rate for E_2 in postmenpausal women of approximately 1,000 L/24 h.[37] Based on an average increment of E_2 of 2 pg/mL during a period of 4 h after each application, our calculations indicate that 0.33 μg

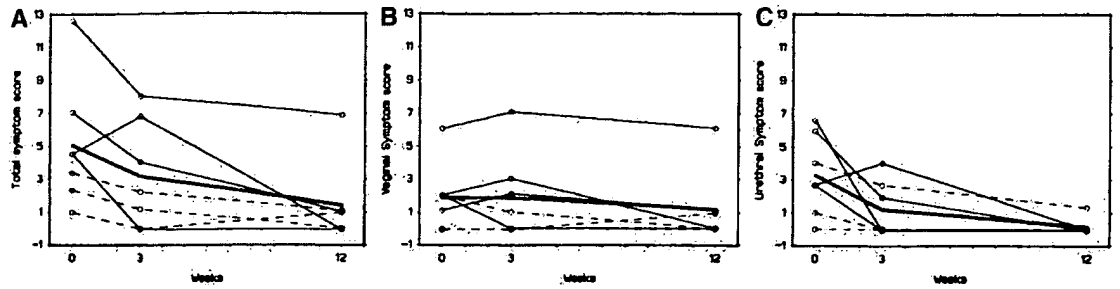

FIG. 3. Total vaginal and urethral symptom severity scores. The dark solid line is the average symptom score at each follow-up time. The lighter lines are individual patient profiles. **A:** Total symptom score by patient and week. Total symptom scores at 12 weeks are significantly different from baseline ($p = 0.01$); although week 3 scores are lower than baseline, they are not significantly different from baseline ($p = 0.10$). **B:** Vaginal symptom score by patient and week. Vaginal symptom scores do not differ significantly ($p = 0.35$). **C:** Urethral symptom score by patient and week. Urethral symptom scores at 12 weeks are significantly different from baseline ($p = 0.01$), but not at 3 weeks ($p = 0.10$).

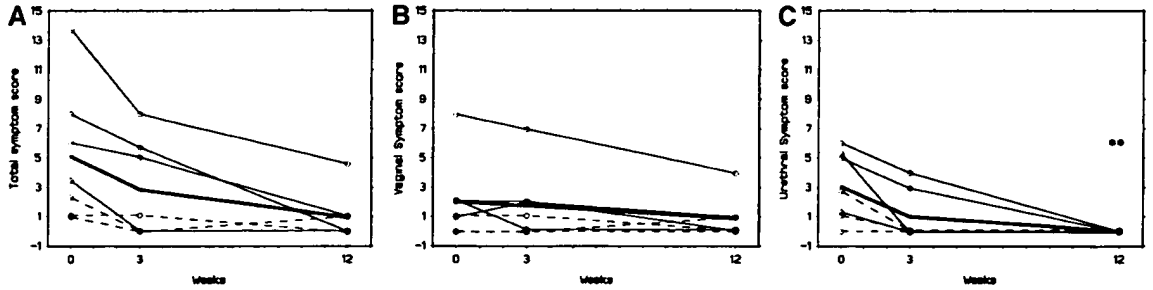

FIG. 4. Total vaginal and urethral symptom bothersome scores. The dark solid line is the average symptom score at each follow-up time. The lighter lines are individual patient profiles. **A:** Total symptom score by patient and week. Week 3 ($p = 0.04$) and week 12 scores ($p = 0.01$) differ significantly from baseline. **B:** Vaginal symptom score by patient and week. Scores do not differ significantly ($p = 0.23$). **C:** Urethral symptom score by patient and week. Week 3 ($p = 0.03$) and week 12 ($p = 0.01$) scores differ from baseline.

of E_2 were absorbed on average. This represents 3.3% of the administered dose.

Estrone

Levels of estrone remained relatively constant during the period of placebo administration and did not exhibit a substantial diurnal variation (Fig. 5B). After administration of E_2 vaginal cream, no acute or chronic increase in estrone levels was observed.

Gonadotropins

Measurement of plasma LH (Fig. 6A) and FSH (Fig. 6B) provides a means to bioassay the amount of estrogen in postmenopausal women. The levels of these hormones can be suppressed with minimal increments in plasma estrogen. We detected no significant decrease in the levels of these hormones with vaginal estrogen cream, thus providing further evidence of the minimal amounts of estrogen absorbed (Fig. 6).

DISCUSSION

This preliminary study suggests that low doses of vaginal E_2 may be used to relieve symptoms of urogenital atrophy and induce objective vaginal changes without substantially increasing plasma E_2 levels. We estimated that approximately 3% of the administered estrogen is absorbed systemically from the vaginal preparation. This results in minor increments of plasma E_2 from 2 to 3 pg/mL for a 4-h period after each twice-weekly dose. The percentages of superficial and parabasal cells return to levels observed in premenopausal women during the early and late follicular phases of their menstrual cycles. Taken together, these data suggest an approach to treatment of urogenital atrophy in survivors of breast cancer that can relieve symptoms without causing a substantial increase in systemic exposure to estrogen.

Inhalent treatment regimens have been developed for patients with asthma, which achieve a predominantly local effect and which reduce systemic glucocorticoid exposure. These studies demonstrate that systemic absorption does occur with use of high local concentrations of steroid, but lower doses minimize this effect. Early studies with vaginal estrogen also demonstrated substantial absorption with high-dose estrogen, particularly when used in patients with very

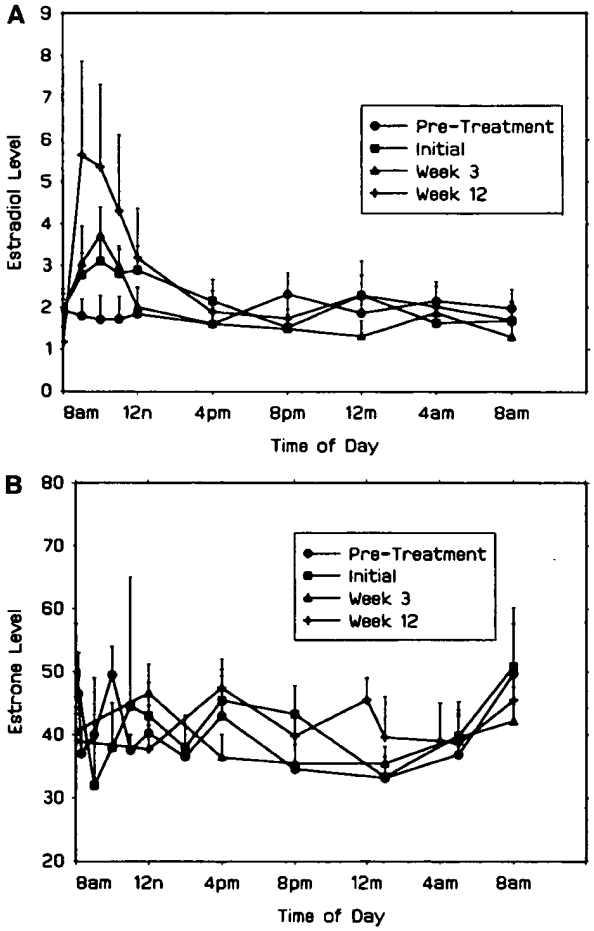

FIG. 5. **A:** Mean estradiol levels by week and time of day. **B:** Estrone levels by week and time of day. Values are mean ± 1 SE. No differences are statistically significant.

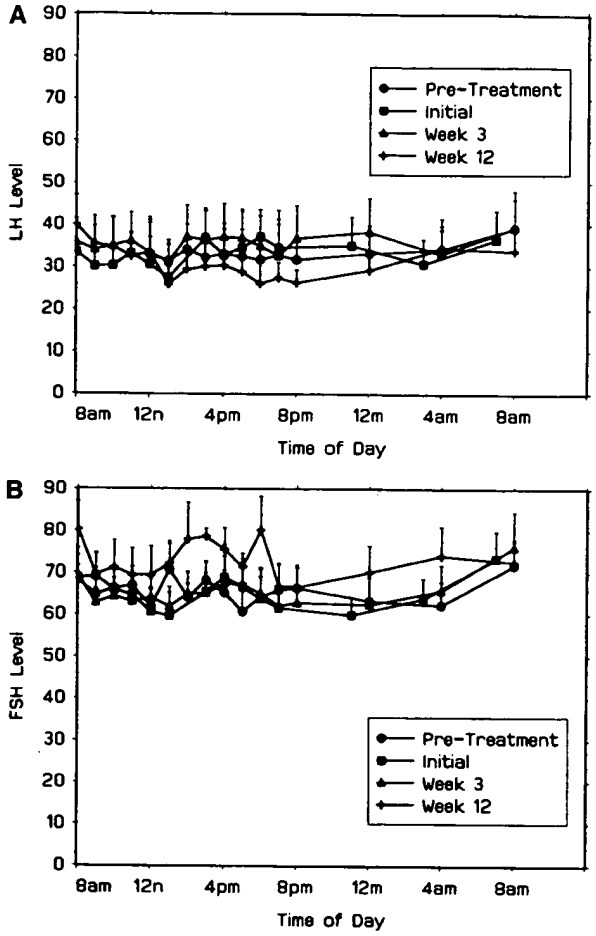

FIG. 6. **A:** Mean luteinizing hormone (LH) levels by week and time of day. **B:** Follicle-stimulating hormone (FSH) levels by week and time of day. Values are mean ± 1 SE. No differences are statistically significant.

atrophic vaginal mucosa.[14] The current preliminary trial suggests that, as with inhaled glucocorticoids, reducing the topical dosage to a minimum allows continued local effect with reduced systemic effects.

The overall goal of the present study is to determine the minimal effective dose of vaginal E_2. No proper dose de-escalation trials have been conducted in the past to determine this endpoint. To our knowledge, all previous trials of vaginal estrogen have used doses that are completely effective in causing vaginal maturation.[38–40] Consequently, it is unknown whether doses substantially less than those used in this study will be equally effective. The experimental design of the current ongoing study is to reduce the vaginal E_2 doses step-wise to 1.25 μg twice weekly and then to use a placebo cream if the 1.25-μg dose is still effective.

Use of the ultrasensitive E_2 bioassay enhanced our ability to detect increments in plasma E_2. Currently available RIAs are not capable of detecting increments as small as 2 pg/mL. Notably, the increments detected in this study were highly reproducible and yet probably too minimal to be significant biologically. Nonetheless, it may be that an even lower amount of E_2 delivered vaginally will be equally effective and not increase plasma E_2 levels. For that reason, we plan to continue with our original experimental design and sequentially examine the effects of lower E_2 dosages. The preliminary results reported here fully justify this approach.

Several other investigators have examined lower doses of vaginal estrogen than the 100-μg dose of E_2 currently recommended. The lowest dose of vaginal E_2 reported to be effective for treating vaginal atrophy is 5 μg/day, delivered via a silastic ring. This device initially releases a "burst amount of E_2" of approximately 200 μg/day.[18] After 1 to 2 weeks, it then releases an average of 5 μg/day. From 5% to 10% is absorbed daily,

or 3.5 μg/week. This is to be compared with absorption from the E_2 cream reported here. Absorption of 0.33 μg of E_2 occurs twice weekly, or 0.66 μg/week. The next lowest dose reported in the literature is 10 μg daily delivered via a vaginal tablet.[23,24] We cannot definitively conclude that the small amounts of E_2 absorbed (i.e., 66 μg/week) in this study do not exert some biologic effects, but this would seem highly unlikely.

A major question regarding previously published studies on vaginal estrogen relates to the precise determination of systemic absorption caused by limitations imposed by assay sensitivity. Studies with the silastic vaginal ring delivery system generally report undetectable E_2 levels before and during therapy.[25] However, standard RIAs are not sufficiently sensitive for such analyses. Measurements of conjugated E_2 metabolites, such as estrone sulfate, in contrast, have detected increments in patients receiving vaginal rings.[40] The lowest dose of vaginal tablet available (10 μg) was studied for evidence of systemic absorption after 2 weeks of treatment.[21] There was evidence of a slight but significant increase in E_2 levels after 14 days of treatment but no suppression of FSH and LH. These observations bring into question the validity of the E_2 assay used.

A key component of our current study was the ability to precisely measure low concentrations of plasma E_2 in postmenopausal women. This is now possible because of a major recent advance: the development of an ultrasensitive E_2 bioassay based on recombinant DNA technology and a yeast expression system. This bioassay enhances sensitivity of the assay by 50- to 100-fold and can detect levels in plasma as low as 0.02 pg/mL.[29–31] As mentioned earlier, our group has conducted studies using this bioassay in women treated with letrozole, an aromatase inhibitor, finding that basal levels of E_2 in postmenopausal women averaged 2 pg/mL and were suppressed to 0.07 pg/mL (95%) with letrozole administration.[30] Comparative measurements on the same samples with a highly sensitive RIA demonstrated basal levels averaging 6 to 8 pg/mL that suppressed to 2 pg/mL (80%). These results suggest that the RIA detects significant "blank" values at low levels of E_2, such as those seen in postmenopausal women. The yeast bioassay, in contrast, is highly specific for E_2, with 0.3% cross-reactivity with estrone. We feel confident that we have indeed measured the true levels of E_2 with this ultrasensitive assay.

Previous studies of vaginal estrogen administration have been limited in several respects. No comprehensive data are available on responses of urethral mucosa, although the slight increases in superficial cells observed in this study are within the ranges expected.[37] No systematic endometrial biopsy data have been obtained to compare acute versus chronic absorption of estrogen.[33,39–42] In general, progesterone withdrawal challenge has been used as a noninvasive means to detect endometrial stimulation. We believe that this method is insufficiently sensitive to assess endometrial hyperplasia. In this study, endometrial biopsies were performed that revealed no evidence of hyperplasia or proliferation. Finally, thin, atrophic vaginal mucosa absorbs E_2 more effectively than does thickened, mature mucosa. The majority of studies have not taken this phenomenon into account. Our study measures E_2 levels during acute administration as well as chronic.

In summary, we report preliminary data from an ongoing study that demonstrate the feasibility of using low-dose vaginal E_2 cream as a method to achieve local effects without substantial systemic absorption. Ultrasensitive assays for E_2 are required to adequately assess this phenomenon. We plan to continue with our original study design to conduct a dose de-escalation protocol and determine minimal effective doses.

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